

**EXHIBIT 2**

**THE SIMANEK ARTICLE:**

**“THE EFFICACY OF GLUCOSAMINE AND CHONDROITIN SULFATE IN  
THE TREATMENT OF OSTEOARTHRITIS: ARE THESE SACCHARIDES DRUGS  
OR NUTRACEUTICALS?”**

## THE EFFICACY OF GLUCOSAMINE AND CHONDROITIN SULFATE IN THE TREATMENT OF OSTEOARTHRITIS: ARE THESE SACCHARIDES DRUGS OR NUTRACEUTICALS?

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This review summarizes recent knowledge on the efficacy of glucosamine (GS) and/or chondroitin sulfate (CS) in the therapy of mild to moderate osteoarthritis (OA). OA, the most common joint disease is a significant source of disability, quality of life impairment and a considerable burden to any health care system. In the Czech Republic, glucosamine sulfate (GS) and chondroitin sulfate (CS) are available both as prescription drugs and as food supplements. Based on available data both are useful in the earlier stages of OA when combined with other modalities such as weight loss and exercises. They appear to relieve pain and improve range of the joint motion. In addition, they also display mild anti-inflammatory effects. However, controversy still exists over their ability to change significantly the natural history of the osteoarthritic joint. This effect is not easy to demonstrate for any other treatment modalities apart from joint replacement. Monitoring the cure efficacy by X-ray has been recently criticised and hence future techniques are anticipated for this reason. Further, long-term oral administration is required to obtain slightly increased levels of GS and/or CS in human blood. Both reviewed saccharides are well tolerated with negligible adverse reactions. In conclusion, the authors suggest that GS and CS should be classified as food supplements only.

### INTRODUCTION

Osteoarthritis (OA) is the most common disorder of the synovial joints in middle aged and older people.<sup>1</sup> It is characterised predominantly by a focal or global loss of articular cartilage, bone changes and an imbalance in inflammatory and non-inflammatory pathways including proteolysis of aggrecans and collagens combined with distortion of their synthesis by chondrocytes.<sup>2</sup> It is a source of great morbidity, impaired quality of life in affected individuals as well as a significant burden to any health care system.<sup>3</sup> It is estimated that more than one third of people over 45 years complain of OA-related symptoms.

The leading symptoms of the OA are pain, stiffness and decreasing functional capacity of the affected joint.<sup>3</sup> The natural history of the disease may vary from a very slow process to a progressive one, where the joint is severely eroded over several months. Fortunately the former is more frequent. On the other hand, spontaneous resolution of a previously osteoarthritic joint has only been mentioned anecdotally.<sup>4</sup> Recently, OA has been interpreted as manifestation of a complex disease with a complicated structure of gene dispositions.<sup>5</sup>

In the past decades, surgical and conservative protocols have been developed to treat osteoarthritis.<sup>6</sup> These are targeted at pain control, inhibition of inflammatory cytokines and proteolytic enzyme activity, free radical release damping, increasing chondrocyte number and function, modification of mechanical conditions in favour of the affected cartilages, etc. However, if the final goal is to fully restore the structural and functional properties of the original tissues, none of the above is adequately reliable.<sup>7</sup> Glucosamine (GS) and chondroitin sulfate (CS) are routinely used in practice. The aim of this paper is to review their efficacy and safety in the treatment of OA.

#### *The natural role of GS and CS in cartilage structure*

Articular cartilages represent a highly organized mixture of chondrocytes, Type II collagen, proteoglycans and water that develop as part of endoskeleton growth and mature under the influence of functional loading.<sup>8</sup> Chondrocytes produce components of the extracellular matrix and regulate the cartilage metabolism similarly to cells in other connective tissues.<sup>9</sup> In this context, the number and functionality of chondrocytes guarantee the anatomical and tribological features of the cartilage.<sup>10</sup> However,

the mitotic and synthetic activities of these cells decline with age.<sup>11</sup>

The macromolecular composition of extracellular matrix, where Type II collagens and aggrecans are the most important and abundant, is a key factor defining the physicochemical properties of the cartilage.<sup>12</sup> Aggrecans are large molecules consisting of a central protein core with several distinct domains and with their different functions.<sup>13</sup> Chondroitin sulfate and the N-terminal hyaluronan-binding (G1) part belong to the most important domains. Glycosaminoglycans are linear acidic polysaccharides containing disaccharide repeat units of D-glucuronate/L-iduronate/D-galactose linked to sulphated *N*-acetylglucosamine/*N*-acetylgalactosamine. Such complexes are naturally synthesized in each joint resulting in high local concentrations.

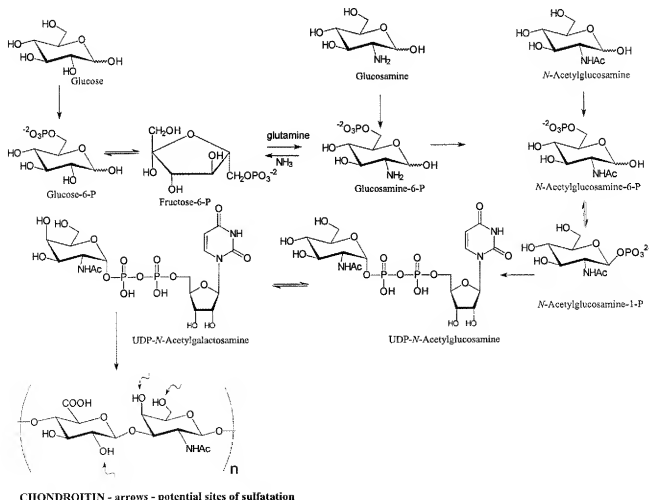
#### Chondroitin sulfate

Chondroitin sulfate consists of an alternating sequence of D-glucuronate and *N*-acetyl-D-galactosamine-4/6-sulfate residues linked through alternating bonds (Scheme 1). It is an essential component of the connective tissue extracellular matrix including the hyaline cartilage, providing its elasticity and other functions. CS belongs to a heteroge-

neous family of glycosaminoglycans with a relatively high molecular weight and charge density.<sup>9</sup> The CS is absorbed in the small intestine in low amounts (<10 %) in the intact form, probably by the mechanism of endocytosis. In the distal gastrointestinal tract, the CS acts as a prebiotic and is degraded by the enzymes in the intestinal flora to di- and monosaccharides.<sup>14</sup> These substances help to maintain constant levels of proteoglycan precursors and also supplement the *N*-acetylglucosamine. Numerous studies have demonstrated the efficacy and availability of CS in experimental and human tissues.<sup>14-16</sup> CS is usually manufactured from bovine or porcine cartilaginous material and also from shark cartilage. Various CS formulations are strongly influenced by the structure and characteristics of raw material origin.<sup>17</sup> The recommended dose for long term usage is 800 mg per day.

#### Glucosamine

GS is an amino monosaccharide, which participates in the constitution of glycosaminoglycans, a major class of extracellular complex polysaccharides. The raw material is derived from chitin, a biopolymer present in the exoskeleton of marine invertebrate animals.<sup>18</sup> There may be significant differences in purity and other phar-



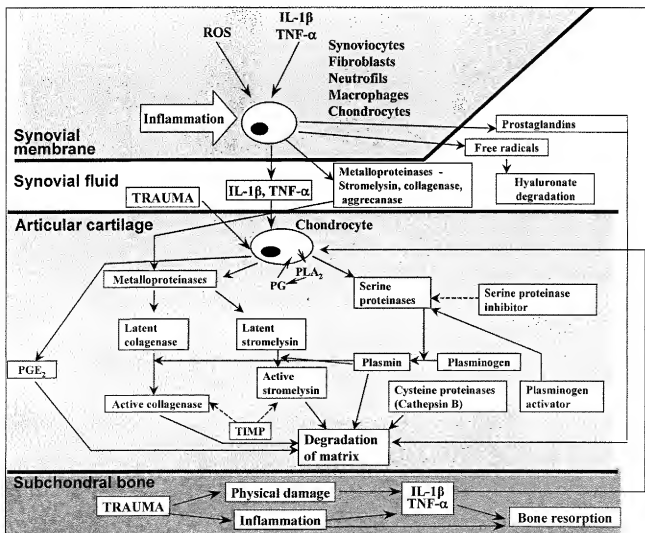
Scheme 1. Biosynthesis of chondroitin.



**Scheme 2.** Glucosamine sulfate (co-crystallized with 2 KCl) and 2-Acetamido-2-deoxy-D-glucopyranoside (*N*-Acetylglucosamine, GlcNAc).

maecutical features among products even though the GS is processed only by a few companies.<sup>19</sup> Glucosamine sulfate, glucosamine hydrochloride and *N*-Acetylglucosamine (Scheme 2) are commonly used alone or as part of the mixtures produced by the pharmaceutical industry. GS is usually taken orally despite having no active intestinal transport.<sup>20,21</sup> The recommended dose for long term usage is 1500 mg per day. According to Anderson et al.<sup>20</sup> daily concentrations of GS in the serum can reach 0.06 mM when a routine dosage of 23.1 mg/kg body weight is administered. It seems unlikely that such low levels would

interfere with sugar metabolism. In contrast to Anderson, other authors recommend special care when GS is administered to patients with diabetes Type II (ref.<sup>22</sup>). In addition, *N*-acetylglucosamine, a metabolic product of GS, enhances basal and fMLP-induced (*N*-formyl-methionyl-leucyl-phenylalanine) motility in neutrophils by modification of serine/threonine residues on cytoplasmatic and nuclear proteins.<sup>23</sup> This O-GSac modification is thought to play a role in the regulation of different signal transductions.



**Fig. 1.** The current concept of osteoarthritic cascade of events (ROS - reactive oxygen species, TIMP - tissue inhibitors of metalloproteinases).

### Suggested therapeutic action for GS and CS

There is suspected chondrocyte insufficiency and/or apoptosis in OA resulting in the inability to remodel effectively the extracellular matrix and repair local cartilage defects.<sup>3</sup> OA is associated with increasing levels of matrix metalloproteinases – collagenases, aggrecanases, stromelysin etc.<sup>24</sup> Simultaneously aseptic inflammation of synovial tissues is present with changes in joint fluid composition that may further impair cartilage metabolism and its wear.<sup>25</sup> Interleukin 1 $\beta$  and TNF- $\alpha$  are considered to be the most prominent inflammatory cytokines to participate in osteoarthritic progression.<sup>2,26</sup> Both induce NO release through mesenchymal cells. NO as well as other free radicals may increase chondrocyte vulnerability and apoptosis. In concert, all these factors strongly distort joint homeostasis in terms of cartilage loss, capsule thickening and a series of ligament, muscle and bone changes (Fig. 1).

Glucosamine and chondroitin sulfate are symptomatic slow-acting drugs for osteoarthritis (SYSADOA). These substances are characterised by both a several week delay in improvement of OA symptoms and by a carryover effect of that improvement.<sup>6</sup> The rationale for their usage is based on a general belief that osteoarthritis is associated with a local deficiency in some key natural substances. Therefore, it is assumed that they work as a "building box" for cartilage extracellular matrix repair.<sup>21,27</sup> Increasing proteoglycan synthesis by chondrocytes has been suggested as another mechanism of CS action.<sup>28</sup> In addition, CS may inhibit the activity of degradative enzymes.<sup>28,29</sup> Implied "anti-osteoarthritic" activities of GS and CS are summarized in the table 1.

### Evidence for GS and CS usage

In fact, a supplementation approach looks rather simplistic against the background of a not as yet fully under-

stood disease pathophysiology. Despite this, many studies have been conducted to demonstrate significant pain relief and functional improvement after GS or CS therapy in OA when compared to either placebo or NSAIDs, using randomized controlled trials (RCTs) and meta-analyses.<sup>19,28,30,31</sup> Unfortunately, the majority of studies were sponsored by the manufacturers and/or have methodological weaknesses.<sup>19</sup> As a result, one may conclude that there is evidence for mild to moderate improvement of symptoms due to GS and CS. On the other hand, this does not exclude the possibility that they bring positive effects to many individuals, and, compared to NSAIDs they have a very low risk of adverse reactions.<sup>32</sup> Nevertheless, many questions remain to be answered.

One of the most representative studies on the influence of CS/GS on knee osteoarthritis was begun in February 2000 at the National Institutes of Health (NIH) in the USA. The patients were randomly assigned to receive either (1) GS alone, (2) CS alone, (3) GS and CS in combination, (4) celecoxib (Celebrex), or (5) a placebo. Overall 13 research centres across the USA participated in this study under the coordination of the University Utah School of Medicine. However, the results of this multicenter and well-designed clinical trial are not available up to the present time.<sup>33</sup>

Controversy still exists over the structure-modifying effect of a GS or CS medication on the cartilage matrix.<sup>34</sup> Long-term use of GS and/or CS has been suggested on the basis of systematic reviews of RCTs or meta-analysis.<sup>35,36</sup> However, the main weaknesses of the analyzed RCTs are the lack of standardization of the OA diagnosis and a questionable outcome assessment. Unsuitability of joint space width measurement has been criticised repeatedly, and future techniques are expected for measurement of the true osteoarthritic regression.<sup>37–39</sup> As a result, the structure-modifying effect still remains unproved for both

**Table 1.** Parameters used for assessment of glucosamine, N-acetylglucosamine and chondroitin sulfate "anti-osteoarthritic" activities *in vitro/in vivo*.

| Parameter                                   | GS | N-AcetylGS | CS | Experiment              |
|---|----|------------|----|-------------------------|
| Blood level of GS                           | ↑  |            |    | <i>in vivo</i>          |
| Blood level of CS                           |    |            | ↑  | <i>in vivo</i>          |
| UDP-N-acetylGS                              | ↑  |            |    | <i>in vitro</i>         |
| NO production                               | ↓  | ↓          |    | <i>in vitro</i>         |
| PLA <sub>2</sub>                            | ↓  |            | ↓  | <i>in vitro/in vivo</i> |
| mRNA of aggrecan and perlecan core proteins | ↑  |            |    | <i>in vitro</i>         |
| Collagenase activity                        | ↓  |            | ↓  | <i>in vitro/in vivo</i> |
| IL-1 $\beta$                                | ↓  | ↓          |    | <i>in vitro</i>         |
| TNF- $\alpha$                               |    | ↓          |    | <i>in vitro</i>         |
| MMP activity                                | ↓  |            | ↓  | <i>in vitro</i>         |
| Proteoglycan synthesis                      |    |            | ↑  | <i>in vitro</i>         |
| COX-2 activity                              |    | ↓          |    | <i>in vitro</i>         |

Table excludes studies with limited statistical significance. ↑ – Decreased/increased, COX-2 – Cyclooxygenase-2, MMP – Matrix metalloproteinase, IL-1 $\beta$  – Interleukin-1 $\beta$ , PLA<sub>2</sub> – Phospholipase A<sub>2</sub>, TNF- $\alpha$  – Tumor necrosis factor  $\alpha$ ,

**Table 2.** Drugs and selected dietary supplements for osteoarthritis on the Czech market.

| Preparation                             | Active components (mg) in pill/sac |
|---|------------------------------------|
| CONDROSULF 400 <sup>a</sup>             | CS (400)                           |
| CONDROSULF 800 <sup>a</sup>             | CS (800)                           |
| DONA <sup>a</sup>                       | GS (1500)                          |
| HYALGAN syringe <sup>a</sup>            | HUNa <sup>b</sup>                  |
| SYNVISC syringe <sup>a</sup>            | HUNa <sup>b</sup> (10/20)          |
| MOBILIN <sup>a</sup>                    | GS (1500), CS (50), CH (50)        |
| ARTRYN <sup>a</sup>                     | GS (800), CS (25), CH (175)        |
| ARTHROSTOP RAPID <sup>a</sup>           | GS (533.3), CS (200)               |
| GELACTIV <sup>a</sup>                   | GS (500), CS (400), CH, MSM (200)  |
| GS-CS-MSM 3000 <sup>a</sup>             | GS (500), CS (200), MSM (300)      |
| GS-CONDRO <sup>a</sup>                  | GS (400), CH (200)                 |
| GS-CONDROFORTE <sup>a</sup>             | GS (800), CH (200)                 |
| PROENZI 3 <sup>a</sup>                  | GS (500), CS (200), MSM (300)      |
| PROENZI PREMIUM FORTE 2700 <sup>a</sup> | GS (500), CS (400)                 |

<sup>a</sup>Drug on prescription, <sup>b</sup>In 1 ml, <sup>c</sup>Food supplement, CS – Chondroitin sulfate, GS – Glucosamine sulfate, HUNa – Sodium hyaluronate, CH – Collagene hydrolysate MSM – Methyl sulfonylmethane

reviewed substances. In addition, long-term monitoring of all patients involved in clinical studies and their appropriate medication may be also difficult.<sup>49</sup> Moreover, different natural histories of the disease and the debatable mechanism of the CS and GS actions have to be considered.

#### Drugs or nutraceuticals?

A plethora of drugs and food supplements consisting of glucosamine and chondroitin sulfate are on the market in the Czech Republic (Table 2). Both GS and CS drugs and supplements are produced under similar if not the same conditions given to GMP, ISO standards, and internal audits. However, food supplements do not need approval from the State Institute for Drug Control. We believe that this mini-review shows that the potential difference between the above products is not significant enough to justify the existence of two controversial terms for one single substance (food supplements vs drugs). Moreover, it may be alleged that extensive prescription of the reviewed substances will not relieve the economic burden of the current treatment strategies for osteoarthritic joints.

#### CONCLUSION

Both CS and GS are clearly defined by their origin, chemical structure and expected efficacy. They exhibit a low risk of adverse effects. Based on the available data there is no rationale to distinguish between drugs and nutraceuticals with regard to the reviewed compounds (providing that the recommended dose and adequate purity are respected). As a result, we prefer the term food supplements to drug. In addition, this paper supports wider usage of the reviewed substances in earlier and moderate stages of osteoarthritis.

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